

CHLOROPLAST PIGMENTS AND THE CLASSIFICATION OF SOME SIPHONALEAN GREEN ALGAE OF AUSTRALIA¹

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With respect to their chloroplast pigments, the siphonaceous green algae of the order Siphonales (class, Chlorophyceae) differ slightly from the common unicellular and multicellular green algae (Strain, 1958, pp. 37, 162, 167). From this standpoint of pigment composition, the Siphonales are not so closely related to the other green algae as all the other uninucleate and multinucleate Chlorophyceae are related to one another (Fritsch, 1935, pp. 60, 368; Smith, 1955, p. 101).

From the nature and the proportions of their chloroplast pigments, the Siphonales are remotely related to the other siphonaceous or coenocytic algae such as the Vaucheriaceae (Strain, 1958, pp. 51, 169), which are alternatively classified as Siphonales (Fritsch, 1935, p. 368) or as Heterosiphonales (class, Xanthophyceae or Heterokontae) (Smith, 1955, p. 177; Taylor, 1960, p. 190). With respect to their pigments, the Heterosiphonales are similar to a group of multicellular filamentous algae known as the Heterotrichales (Tribonemataceae), also of the class Heterokontae (Smith, 1955, p. 174; Strain, 1951, p. 254, 1958, p. 169). This relationship, based on pigments, supports the contention that the Heterosiphonales (Vaucheriaceae) and the Heterotrichales (Monociliaceae and Tribonemataceae) belong to the same class (Heterokontae) (Smith, 1955, p. 166). With respect to pigments, *Dichotomosiphon tuberosus* (A.Br.) Ernst, a fresh-water, siphonaceous species, is related to the Siphonales (Strain, 1958, pp. 37, 167) (predominantly marine species), not to the siphonaceous Heterosiphonales (predominantly fresh-water species) (Fritsch, 1935, p. 369; Smith, 1955, pp. 101, 115; Taylor, 1960, p. 190).

Like all the common green algae, and the higher plants as well, the species of the Siphonales examined thus far contained chlorophyll *a* plus chlorophyll *b*. The heterosiphonalean and heterotrichalean species contained chlorophyll *a* unaccompanied by other chlorophylls (Strain, 1958, pp. 37, 51, 167, 169). Like the common green algae, the coenocytic species of the order Siphonales contained the principal xanthophylls neoxanthin, violaxanthin and lutein plus traces of zeaxanthin. They also contained an additional xanthophyll, called siphonaxanthin, and an ester of this pigment called siphonein. The heterosiphonalean and the heterotrichalean species contained a group of unnamed xanthophylls not observed elsewhere (Allen *et al.*, 1964; Strain, 1958, p. 51). The green algae of the order Siphonales contained much more α -carotene than β -carotene. The algae of the heterokontae groups contained principally β -carotene. *Dichotomosiphon* contained the same chlorophylls and xanthophylls found in the Siphonales, but the principal carotene was the β -isomer (Strain, 1958, p. 167).

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The siphonalean green algae available for the earlier studies (Strain, 1958, p. 167) included about 24 varieties and species. They represented 9 genera and 5 families. Except for *Dichotomosiphon*, obtained from Lake Michigan, these species were indigenous to the coastal waters of California, Hawaii and Puerto Rico.

MATERIALS AND OBSERVATIONS

As an extension of the earlier studies, the pigments of several Australian species of siphonaceous, green algae have now been isolated and compared with the pigments described before (Strain, 1958). The organisms were collected at various remote locations in the Southern Hemisphere (Womersley, 1959) at low tide during the spring months, August through November, 1963. They were examined soon after collection. The pigments were separated and compared by chromatography in columns of powdered sugar with petroleum ether plus 0.5% *n*-propanol as the wash liquid (Strain, 1958, p. 27). In the United States the powdered sugar is sold as Confectioners Powdered Sugar. In Australia it is available as Soft Icing Mixture and as Icing Sugar. The carotenes were separated in columns of activated magnesia [Micron Brand magnesium oxide No. 2641 (Strain, 1958, p. 30) also sold as Sea Sorb 43 by Fisher Scientific Company]. Before the columns were packed, the magnesia was mixed with twice its weight of heat-treated siliceous earth (Celite 545).

The individual pigments with their sequence (top to bottom) and color in the columns of powdered sugar were:

Siphonaxanthin, red-orange
Neoxanthin, light yellow
Violaxanthin, light yellow
Siphonein, red-orange
Chlorophyll *b*, yellow-green
Lutein $\pm$ zeaxanthin, yellow
Chlorophyll *a*, green
 α -Carotene + β -carotene, yellow

The location of siphonein was previously reported below the chlorophyll *b* due to mislabelling of the chlorophyll *b* and siphonein zones (Strain, 1958, p. 38). Separate chromatographic experiments also revealed that siphonein and fucoxanthin, the principal xanthophyll of diatoms and brown algae, are adsorbed together in the sugar columns. Both pigments form zones of nearly the same color above the chlorophyll *b* zone. When adsorbed together in sugar columns, these two xanthophylls are incompletely separated, even after prolonged washing with the petroleum ether-propanol solvent. Reaction of the fucoxanthin, in ether solution, with concentrated hydrochloric acid, to yield a blue color in the acid layer, serves to distinguish the fucoxanthin from the siphonein, which does not yield a blue color.

The siphonalean algae that were examined are listed in Table I along with the regions where they were collected.

All the species included in Table I yielded much more α -carotene than β -carotene. All these species except *Caulerpa filiformis* contained siphonaxanthin and siphonein in addition to the other chloroplast pigments found in green algae and higher

plants (Strain, 1958, pp. 136, 157, 162). They yielded these pigments in the chromatographic sequence indicated above. *Chlorodesmis comosa* yielded very little of the siphonaxanthin and siphonein.

Because of the absence of siphonaxanthin and siphonein in *Caulerpa filiformis*, analyses were repeated several times. The organism was also collected from two different stands on neighboring rocks, but the siphonaxanthin and siphonein, found in other species of *Caulerpa* as well as in all the other species of the Siphonales, could not be detected.

TABLE I

Algae of the order Siphonales, whose chloroplast pigments were determined, and the locations where they were collected in Australia

Bryopsidaceae	
<i>Bryopsis</i> sp.	Cottesloe Beach (Perth)
Caulerpaceae	
<i>Caulerpa cupressoides</i> (West) C.Ag.	Heron Id. (Gt. Barrier Reef)
<i>Caulerpa distichophylla</i> Sonder	Cottesloe Beach (Perth)
<i>Caulerpa filiformis</i> (Harv.) C.Ag.	Cronulla Beach (Sydney)
<i>Caulerpa lentillifera</i> J. Ag.	Redcliff (Brisbane)
<i>Caulerpa racemosa</i> (Forssk.) J.Ag.	Heron Id. (Gt. Barrier Reef)
<i>Caulerpa racemosa</i> var. <i>lactevirens</i> (Mont.) Weber-van Bosse	Pt. Peron (Freemantle)
<i>Halimeda discoidea</i> Decaisne	Heron Id. (Gt. Barrier Reef)
Codiaceae	
<i>Chlorodesmis comosa</i> Harv. & Bail.	Heron Id. (Gt. Barrier Reef)
<i>Codium duthieae</i> Silva	Pt. Peron (Freemantle)
<i>Codium fragile</i> (Suring) Hariot	Hungry Pt. Cronulla (Sydney)
<i>Codium lucasii</i> Setch.	Cronulla (Sydney)
<i>Codium lucasii</i> Setch.	Redcliff (Brisbane)
<i>Codium spongiosum</i> Harv.	Heron Id. (Gt. Barrier Reef)

Cladophoropsis herpestica (Mont.) Howe (Valoniaceae), collected at Redcliff (Brisbane), yielded the pigments characteristic of the uninucleate green algae. Siphonaxanthin and siphonein were absent, and β -carotene was the principal carotene isomer. These results conform to those obtained with other species of the Valoniaceae, Boodleaceae and Anadyomenaceae (order, Siphonocladales) (Strain, 1958, p. 164).

CONCLUSIONS

On the basis of the species available thus far, siphonaxanthin and siphonein are constituents of siphonalean green algae indigenous to remote regions both of the Northern and Southern Hemispheres. The absence of these two pigments in *Caulerpa filiformis* indicates that siphonaxanthin and siphonein are not definitive characters for the classification of all the Siphonales. The preponderance of α -carotene relative to β -carotene is without exception in the marine Siphonales. The fresh-water *Dichotomosiphon* is an exception (Strain, 1958, p. 157).

The wide distribution of siphonaxanthin and siphonein in the species examined (Table I) indicates that the Siphonales comprise a distinct group. This conclusion

is supported by the preponderance of α -carotene over β -carotene in all the marine species.

The absence of siphonaxanthin and siphonein and the preponderance of β -carotene in *Cladophoropsis herpestica* agree with earlier analyses of other species of the Siphonocladales (Strain, 1958, p. 164). This result indicates that the Siphonales stand apart from the Siphonocladales.

The universal occurrence of chlorophyll *b* along with chlorophyll *a* supports the view that the Siphonales and the Siphonocladales are major divisions of the Chlorophyceae. This relationship is supported by the occurrence of the same xanthophylls, namely, lutein, violaxanthin and neoxanthin, in the Siphonales, in the Siphonocladales and in all the other species of the Chlorophyceae.

There appears to be no relationship among the occurrence of particular chloroplast pigments, the calcification of the organisms and the composition of the structural polysaccharide in the marine Siphonales (Feldmann, 1946; Fritsch, 1935, p. 368; Miwa *et al.*, 1960; Smith, 1955, p. 12).

The occurrence of chlorophyll *a* and β -carotene in the *Siphonales* and in the *Heterosiphonales* indicates a basic relationship between these two groups. This relationship is akin to that existing among all autotrophic organisms in which oxygen production is always associated with the presence of chlorophyll *a* and α - or β -carotene (Strain, 1951, 1958, p. 83, 1964).

In conjunction with the earlier pigment studies (Strain, 1958), the current results support the classification of the Heterosiphonales with the Heterotrichales in a class apart from the Chlorophyceae.

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SUMMARY

1. The chloroplast pigments of twelve species of siphonalean green algae native to the marine waters of Australia were isolated by chromatography.
2. All these siphonalean species but one contained the same pigments found in the siphonalean Chlorophyceae of the Northern Hemisphere.
3. *Caulerpa fliformis* lacked the siphonaxanthin and siphonein associated with the chlorophylls and carotenoids in the other Siphonales, but it contained a preponderance of α -carotene relative to β -carotene as did all the other Siphonales.

4. These observations indicate that the Siphonales are significantly different from the other green algae, but they are more closely related to the Chlorophyceae than to any other algal group.

5. The pigment distribution supports the classification of the Siphonales with the Chlorophyceae, the Heterosiphonales (Vaucheriaceae) and the Heterotrichales (Tribonemataceae) with the Xanthophyceae or Heterokontae.

LITERATURE CITED

- ALLEN, M. B., L. FRIES, T. W. GOODWIN AND D. M. THOMAS, 1964. The carotenoids of algae: pigments from some cryptomonads, a heterokont and some Rhodophyceae. *J. Gen. Microbiol.*, **34**: 259-267.
- FELDMANN, J., 1946. Sur l'hétéroplaste de certaines Siphonales et leur classification. *C. R. Acad. Sci.*, **222**: 752-753.
- FRITSCH, F. E., 1935 (Reprinted 1961). The Structure and Reproduction of the Algae. Vol. I, pp. xvii + 791, 245 figs., University Press, Cambridge.
- MIWA, T., Y. IRIKI AND T. SUZUKI, 1960 (Pub. 1961). Mannan and xylan as essential cell wall constituents of some siphonous green algae. *Colloq. Intern. Centre Nat'l. Rech. Sci.*, (Paris) No. 103: 135-144 (English).
- SMITH, G. M., 1955. Cryptogamic Botany. Vol. I, pp. vii + 546, 311 figs., McGraw-Hill Book Co., New York.
- STRAIN, H. H., 1951. Manual of Phycology, pp. 243-262. Smith, G. M., ed., Chronica Botanica Co., pp. xi + 375, 48 figs., Waltham, Mass.
- STRAIN, H. H., 1958. Chloroplast Pigments and Chromatographic Analysis. 32nd Annual Priestley Lectures, pp. 180, 41 figs., Pennsylvania State University, University Park.
- STRAIN, H. H., 1964. Chloroplast pigments of various plants. *Argonne National Laboratory Reviews*, Vol. 1, No. 3, 6-7.
- TAYLOR, W. R., 1960. Marine Algae of the Eastern Tropical and Subtropical Coasts of the Americas. Pp. xi + 870, 14 photos, 80 pl., University of Michigan Press, Ann Arbor.
- WOMERSLEY, H. B. S., 1959. The marine algae of Australia. *Botan. Rev.*, **25**: 545-614.